

## High morbidity and mortality in cystic fibrosis patients compound heterozygous for 3905insT and $\Delta$ F508

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*High morbidity and mortality in cystic fibrosis patients compound heterozygous for 3905insT and  $\Delta$ F508. A. Schibler, I. Bolt, S. Gallati, M.H. Schöni, R. Kraemer. ©ERS Journals Ltd 2001.*

**ABSTRACT:** Genotype-phenotype association in cystic fibrosis (CF) is difficult because of heterogeneous disease expression. The genotype-phenotype correlation for the 3905insT mutation in comparison to  $\Delta$ F508 was studied here.

Thirty CF patients compound heterozygous for 3905insT were compared to clinical presentation of matched patients homozygous for  $\Delta$ F508 (1960–1997). Sweat tests, age at diagnosis, at death and at onset of *Pseudomonas aeruginosa* colonization were analysed. Crispin-Norman scores and pulmonary function forced expiratory volume in one second (FEV<sub>1</sub>) determined severity of lung disease. Twenty-five of the patients with 3905insT had  $\Delta$ F508 as a second mutation and five had another rare mutation.

At the age of 15 yrs, 60% of patients with 3905insT had an FEV<sub>1</sub> < 60% predicted in comparison to 25% of patients with  $\Delta$ F508 ( $p < 0.05$ ). Age at death and cumulative survival rate was significantly lower ( $p < 0.05$ ) in the 3905insT than in the  $\Delta$ F508 group (20.3 and 24.0 yrs, respectively). Age at onset of *P. aeruginosa* colonization was not different in the study groups. Sweat chloride concentrations were lower in patients homozygous for  $\Delta$ F508 ( $105.63 \pm 15.3 \text{ mmol} \cdot \text{L}^{-1}$ ) than in patients with 3905insT ( $119.9 \pm 22.1 \text{ mmol} \cdot \text{L}^{-1}$ ) ( $p < 0.05$ ).

Patients compound heterozygous for 3905insT have similar high morbidity and mortality to patients homozygous for  $\Delta$ F508.

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Striking heterogeneity in the initial presentation, clinical course, and prognosis of patients with cystic fibrosis (CF) has been noted for many decades. CF is caused by defects in the CF transmembrane conductance regulator (CFTR) gene, which encodes for a protein that functions as a chloride channel regulated by cyclic adenosine monophosphate (cAMP). Defects in the CFTR gene cause abnormal chloride conductance across the apical membrane of epithelial cells in the airways, pancreas, intestine and sweat glands, resulting in a progressive lung disease, pancreatic dysfunction and elevated sweat electrolytes [1].

The most common mutation,  $\Delta$ F508, is associated with an early presentation, pancreatic insufficiency, and significant progressive lung disease [2–8]. There are other studies showing good association between  $\Delta$ F508 and pancreatic insufficiency, but not with severity of lung disease [9–12]. Reviewing the literature carefully, there are reports showing good genotype-phenotype association [13, 14]. Comparison of the long-term clinical course of CF in patients with different genotypes is difficult because therapy modalities have improved consistently over the last few years and therapy compliance varies from patient to patient. Recently a novel insertion mutation in exon 20 of the CFTR gene, 3905insT, was discovered [8]. This mutation accounts for the second most common (4.8%) CFTR gene mutations in Switzerland [15]

and among the Amish population in USA, its frequency is reported as high as 16.9% [16].

This report describes the results of a study in which 30 patients compound heterozygous for 3905insT are compared, in respect to their phenotype, to aged and sex matched patients homozygous for  $\Delta$ F508. Clinical parameters such as onset of chronic *Pseudomonas aeruginosa* infection, lung involvement evaluated by chest radiograph score and pulmonary function tests (PFTs), relative underweight and sweat electrolyte levels were examined.

### Patients and methods

#### Patients

Clinical findings and lung function data in the time period 1960–1997 were collected retrospectively from hospital charts of 30 patients (19 females and 11 males) carrying the mutation 3905insT. Twenty-five of these patients showed compound heterozygosity for  $\Delta$ F508 and 3905insT, two patients heterozygosity for R553X and 3905insT, one patient for 1717-G→A and 3905insT, one patient for R347P and 3905insT and one patient with an unknown mutation and 3905insT. Each patient compound heterozygous for 3905insT was matched with a patient homozygous for  $\Delta$ F508 of

the same age and sex. Therefore, the matched patient homozygous for  $\Delta F508$  had to be born within 6 months of the corresponding patient with 3905insT. All patients in the two groups were regularly followed at the CF centre of the children's hospital, Bern, by the same staff, and were ethnically native Swiss, mostly of Celtic and Germanic origin.

#### Clinical assessment

Typical clinical symptoms and/or family history of CF together with at least two abnormal sweat tests (STs) measuring sweat sodium and chloride concentrations, confirmed the diagnosis. For each patient, two main symptoms leading to the diagnosis of CF were defined (failure to thrive, respiratory symptoms, meconium ileus, family history). Relative underweight of the patients during the observation period was described using SD scores and normal values obtained by PRADER *et al.* [17]. The presence or absence of pancreatic insufficiency was determined on the basis of need for pancreatic enzyme supplementation to control steatorrhoea. The faecal fat balance was evaluated by collecting stool samples pooled over a 3 day period and analysing stool fat content according to the method of VAN DER KAMER *et al.* [18]. PFTs were performed with a whole-body plethysmograph (Jaeger®, Würzburg, Germany). Vital capacity (VC) and forced expiratory volume in one second (FEV<sub>1</sub>) were measured and values are expressed as per cent predicted of normal values for age, sex and height [19]. Based on the present authors' experience, a reduction of the FEV<sub>1</sub> <60% pred at the age of 12 yrs indicates a more severe clinical course. To evaluate the morbidity of the CF patients, therefore, a cut-off level of <60% pred for VC and FEV<sub>1</sub> was chosen as an event time for statistical analysis. Lung function assessment was generally possible after the age of 6 yrs. Pulmonary involvement was also established by chest radiography using the Chrispin-Norman [20] score. The onset of chronic *P. aeruginosa* colonization was defined as continuous culture of the bacteria from sputum for >6 months [21, 22].

#### Genotyping of patients

The  $\Delta F508$  mutation, present in ~70% of the CF patients, is a 3-base deletion in the CF gene that results in a loss of a single phenylalanine residue at position 508 of the CFTR protein ( $\Delta F508$ ). The 3905insT is a frameshift mutation located in exon 20 leading to a stop 6 codons after the insertion of one T after nucleotide 3905. R553X and K710X are nonsense mutations in exon 11 and 13, respectively, whereas R347P is a missense mutation in exon 7. The 1717-1 G→A, a 3'splice acceptor site mutation in intron 10 leads to a messenger ribonucleic acid (mRNA) lacking in exon 11 [23]. Total deoxyribonucleic acid (DNA) was extracted from peripheral blood lymphocytes according to standard protocols; 50–200 ng DNA was used in each polymerase chain reaction (PCR) and cycled under standard conditions.

The mutation screening was performed by amplifying exon 7, 10, 11, 13 and 20 by PCR using intronic primers flanking the respective exons. The amplification products were cut with a restriction enzyme and further analysed for sequence variants by a modified single strand conformation polymorphism (SSCP) method based on a two buffer system [24]. To identify the mutations, the PCR products showing a mobility shift on the SSCP gel were sequenced using an abi373A system.

#### Statistics

Group means of annual relative underweight, lung function parameter and chest radiograph score were compared using the two tailed Mann-Whitney U-test allowing for non-normal distribution and small group sizes. Because of small numbers of patients and a large age range at diagnosis and death, a median was used to describe the results. The Spearman rank correlation coefficient was defined for correlation analysis. The cumulative incidence of chronic *P. aeruginosa* infection was calculated according to PEDERSEN *et al.* [21]. Kaplan-Meier survival curves were used to summarize the time to death experience, to *P. aeruginosa* onset and to the decline in lung function. Survival or probability curves were then compared using a log rank test statistic. In all cases, a  $p < 0.05$  was considered statistically significant.

#### Results

DNA from 60 patients was analysed, of which 30 patients were compound heterozygous for 3905insT and 30 patients homozygous for  $\Delta F508$ . Patients compound heterozygous for 3905insT were diagnosed at a similar age as patients homozygous for  $\Delta F508$  (table 1). No difference was found in the symptoms leading to the diagnosis in the two groups. In the 3905insT group there were 17 patients with failure to thrive, seven with respiratory symptoms, two with *meconium ileus* and four with a positive family history. Fifteen patients of the  $\Delta F508$  group showed failure to thrive, 11 presented with respiratory problems, three with *meconium ileus* and four had a positive family history for CF. The total incidence of *meconium ileus* for both groups was 8%. All patients of both groups showed steatorrhoea at the time of diagnosis and were supplemented with pancreatic enzymes.

Figure 1 shows a progressive reduction in lung function (VC and FEV<sub>1</sub>) over the observation period. Patients compound heterozygous for 3905insT showed a significantly ( $p < 0.05$ ) earlier decline of VC and FEV<sub>1</sub> in comparison to patients homozygous for  $\Delta F508$  using cumulative probability and a cut-off level of 60% pred for VC and FEV<sub>1</sub> (fig. 2). At the age of 15 yrs, 60% of patients with 3905insT had an FEV<sub>1</sub> <60% pred in comparison to 25% of patients with  $\Delta F508$  ( $p < 0.05$ ). Lung involvement, established by the Chrispin-Norman [20] score showed, in both groups, a significant positive correlation between the score and age ( $r = 0.589$  for the  $\Delta F508$  group and

Table 1. – Sweat tests, age at diagnosis, at death and at onset of *Pseudomonas aeruginosa* (PA) colonization in different genotypes

	ΔF508	3905insT	Significance
Age at diagnosis yrs	0.3(0–18)	0.25(0–3.6)	NS
Sweat sodium mmol·L <sup>-1</sup>	83.5 ± 16.5	87.9 ± 18.8	NS
Sweat chloride mmol·L <sup>-1</sup>	105.6 ± 15.2	120.0 ± 22.1	p < 0.05
Age at PA onset yrs	7.4 ± 6.6	8.4 ± 6.6	NS
Age at death yrs	24.04(18.4–27.1)	20.25(8.8–32.6)	p < 0.05
Current age yrs	19.4(2.3–36.6)	18.8(2.7–37.3)	NS

Data are presented as median (range) or mean ± SD. Thirty subjects were included in each group (ΔF508 and 3905insT; six died from group ΔF508 and 12 died from group 3905insT). NS: nonsignificant.

r = 0.374 for the 3905insT group, p < 0.05 for both groups). No statistically significant difference in radiograph scores between groups could be found at any age.

In figure 3, the relative underweight for both CF groups is shown separately for survivors and non-survivors. Nonsurvivors compound heterozygous for 3905insT showed significantly higher relative underweight than survivors (p < 0.05), whereas in patients homozygous for ΔF508, no difference between survivors and non-survivors was found. There was no positive correlation between lung involvement assessed by lung function and underweight.

On-set of chronic *P. aeruginosa* colonization was observed at a median age of 7.4 yrs in the 3905insT

group and at 8.4 yrs in the ΔF508 group (table 1). The cumulative incidence of *P. aeruginosa* infection for the two groups at the time of the study was similar, as shown in figure 4. The sweat test showed significantly lower sweat chloride concentration in patients homozygous for ΔF508 (table 1), whereas sweat sodium concentration did not differ between the two groups.

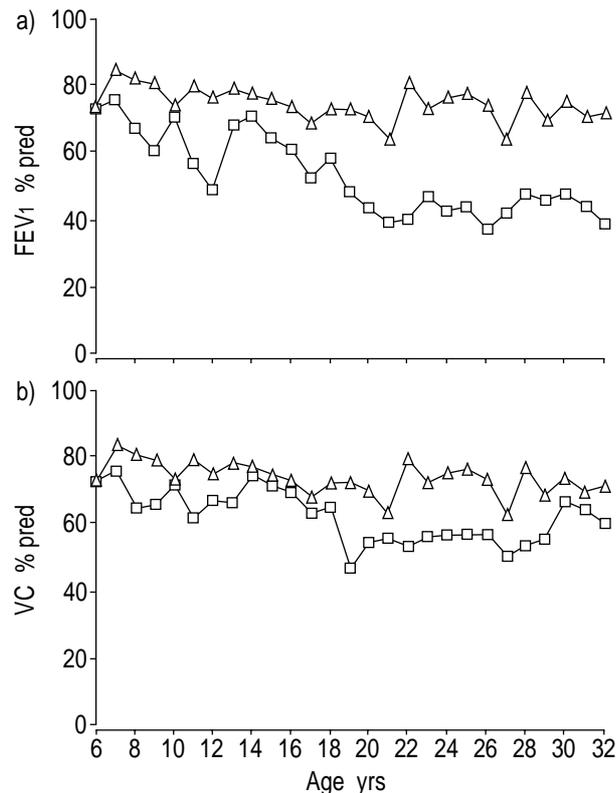


Fig. 1. – Lung function parameters (percentage predicted) a) forced expiratory volume in one second (FEV<sub>1</sub>) and b) vital capacity (VC) are shown for patients compound heterozygous for 3905insT (□) and homozygous for ΔF508 (Δ).

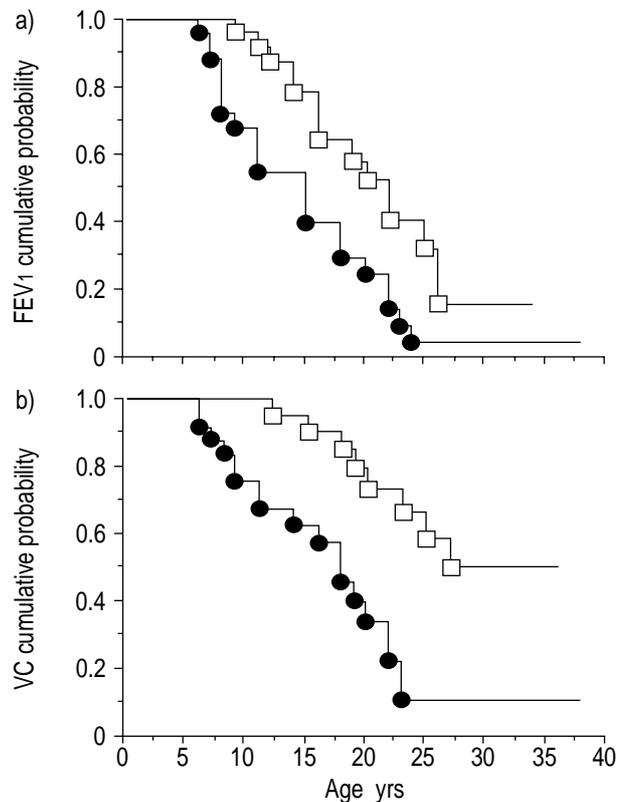


Fig. 2. – Changes of pulmonary function during observation period. Using a log rank test (Kaplan-Meier survival curves), a decline of a) forced expiratory volume in one second (FEV<sub>1</sub>) and b) vital capacity (VC) under 60% predicted was taken as a critical event during the observation period. The graph shows for the two groups, patients homozygous for ΔF508 (□) and patients heterozygous for 3905insT/ΔF508 (●), the estimated cumulative probability having FEV<sub>1</sub> and VC under 60% pred. Patients heterozygous for 3905insT showed a significantly earlier decline in lung function assessed by FEV<sub>1</sub> and VC (p < 0.001). The 25th percentile for FEV<sub>1</sub> was 16 yrs for patients homozygous for ΔF508 and 8 yrs for patients compound heterozygous for 3905insT. The probability status of the 25th percentile for VC was 20 yrs in the ΔF508 group and 11 yrs in the 3905insT group.

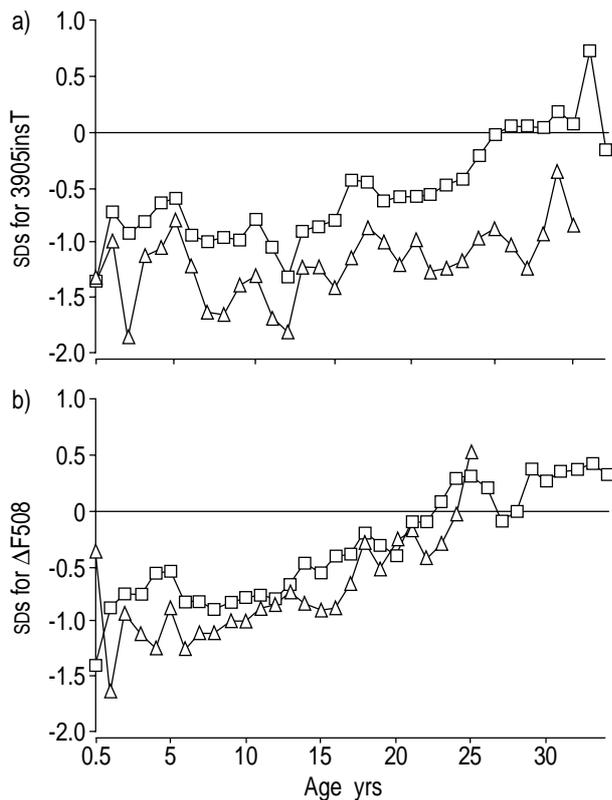


Fig. 3. – Mean relative underweight of cystic fibrosis patients a) compound heterozygous for 3905insT and b) homozygous for  $\Delta$ F508 using standard deviation scores (SDs) and normal values.  $\square$ : mean of all survivors of each group;  $\triangle$ : all nonsurvivors. In the 3905insT group, the relative underweight of the nonsurvivors during the observation period was significantly higher (lower SDs scores) than that of the survivors ( $p < 0.05$ ). In the  $\Delta$ F508 group such a difference between survivors and nonsurvivors was not found ( $p = \text{NS}$ ).

Twelve patients of the 3905insT group were dead at the time of investigation (table 1) and median age at death was 20.3 yrs. In the  $\Delta$ F508 group, six patients died and median age at death was 20.0 yrs. The cumulative survival in the 3905insT group was

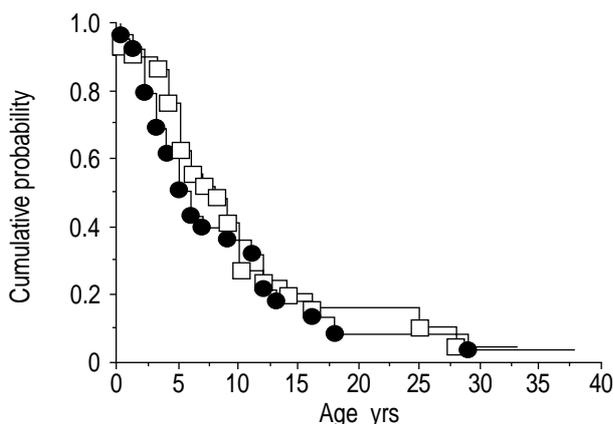


Fig. 4. – Cumulative probability of *Pseudomonas aeruginosa* colonization. No difference ( $p = \text{NS}$ ) between patients heterozygous for 3905insT ( $\bullet$ ) and patients homozygous for  $\Delta$ F508 ( $\square$ ) could be found using Kaplan-Meier survival curves.

significantly lower ( $p < 0.01$ ) as shown in figure 5. An early divergence in the survival curves for the two groups appears, but then similar curves between 20–30 yrs are observed. All patients died because of respiratory failure.

## Discussion

Association between genotype and phenotype in patients suffering from CF is becoming more evident and KEREM and KEREM [25] give an excellent review. Initial reports looking at genotype-phenotype association suggested that the most common mutation,  $\Delta$ F508, was associated with an early presentation, pancreatic insufficiency, and severe progressive lung disease [7]. Several subsequent reports have shown close association between  $\Delta$ F508 and pancreatic insufficiency [3, 4]. However, wide variations of pulmonary disease severity, even in patients homozygous for  $\Delta$ F508, makes it difficult to establish a good genotype-phenotype association.

### Morbidity and mortality

In the present study, an attempt was made to assess morbidity (PFT, on-set of *P. aeruginosa* colonization, relative underweight, STs, radiograph scores) and mortality (survival) in patients compound heterozygous for 3905insT versus patients homozygous for  $\Delta$ F508. Patients compound heterozygous for 3905insT showed a more severe long-term course regarding pulmonary disease (figs. 1 and 2). Patients of the 3905insT group showed an earlier on-set and higher prevalence of *P. aeruginosa* colonization, but the number of investigated patients ( $n = 30$ ) in this study was too small to become statistically significant. It has previously been shown [26] that relative underweight is usually in accordance with the radiograph score, confirming the experience of NEIJENS *et al.* [27] that lung involvement is often linked with a decrease in body weight and hence survival. In the present

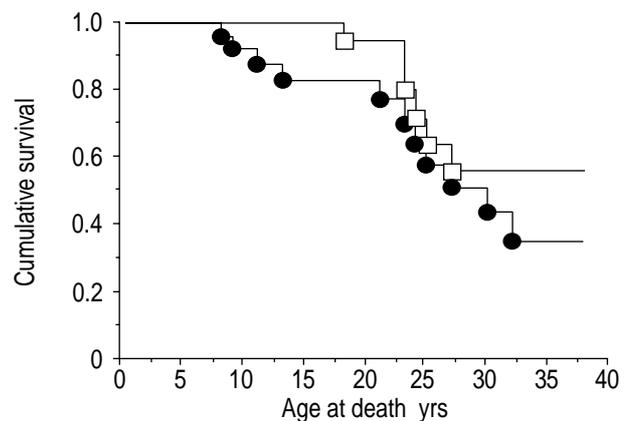


Fig. 5. – Cumulative survival of patients heterozygous for 3905insT ( $\bullet$ ) and patients homozygous for  $\Delta$ F508 ( $\square$ ). Survival is significantly lower in the 3905insT group ( $p < 0.05$ ).

study, however, it was shown that lung function decreases continuously after the age of 10 (fig. 1) while underweight normalizes (fig. 3). All nonsurvivors (12 patients) in the 3905insT group showed significantly lower underweight compared to the survivors, whereas the nonsurvivors of the  $\Delta F508$  group showed no difference in their underweight from the  $\Delta F508$  survivors. Some of these contradictory results could be explained by a negative survival effect [28]. Factors contributing for such an effect are length of observation, late referrals to the CF clinic or difference in treatment modalities. However, all patients were followed-up in the same clinic by the same staff and late referrals do not exist due to the centralized CF caring programme in the Swiss health system. All patients of the two groups showed pancreatic insufficiency and, therefore, pancreatic insufficiency is probably not the primary cause for the differences of relative underweight found in survivors and nonsurvivors in the 3905insT group. A possible explanation is pulmonary cachexia. The mortality rate in the 3905insT group was significantly higher. The total incidence of *meconium ileus* (8%) was low if compared to the results from the United States Cystic Fibrosis Foundation Data Registration [29], presumably due to the wide population specific scattering of CF mutations in Switzerland.

#### Limits of the method

The study was based on hospital charts in the period 1960–1997. The set of data was not complete for all of the investigated clinical parameters, but the level of missing data was similar in both study groups. Changes in treatment modalities during such a large observation period makes comparison difficult. In the present study, however, all the investigated patients were followed at the same CF centre by the same medical staff. The study included a homogenous collective of patients ethnically originating from Switzerland. In a smaller group of CF patients, the genotype-phenotype association of the first 13 patients with the 3905insT [8] have been previously described. The present study was made to test these findings concerning morbidity and mortality after having studied the long-term clinical course. In accordance with previous results, this study concludes that the 3905insT mutation predicts a similar severe clinical course to the  $\Delta F508$ .

At present, the 3905insT mutation is the second most common mutation in Switzerland. Its incidence among the Swiss CF population is 4.8%, and 16.9% in the ethnically Swiss originated Amish population in America. A clear association between genotype and phenotype could be demonstrated. There are few studies demonstrating such an association between genotype and lung disease [6, 7, 9, 12, 25, 30]. JOHANSEN *et al.* [3] describe earlier on-set of *P. aeruginosa* colonization in patients homozygous for  $\Delta F508$  compared to patients with heterozygosity for  $\Delta F508$ . Despite this fact, caution is advisable in interpreting mutation effects and in predicting the clinical course of individual patients with the 3905insT

mutation. One patient compound heterozygous for 3905insT/R347P showed normal lung function tests at the age of 37 yrs, no *P. aeruginosa* colonization, normal body weight and a normal chest radiograph. The patient's sweat test was positive with sodium of  $130 \text{ mmol}\cdot\text{L}^{-1}$  and chloride of  $144 \text{ mmol}\cdot\text{L}^{-1}$ . In comparison to compound heterozygosity for 3905insT and  $\Delta F508$ , the R347P mutation, together with 3905insT, seems to play a dominant role leading to a less severe clinical course. The other four patients with compound heterozygosity for 3905insT and 1717-1G→A, R553X and K710X, respectively, did not show a different clinical course from patients with 3905insT/ $\Delta F508$ . In addition, environmental factors such as different medical therapies, compliance, early severe infection, and passive exposure to tobacco and other pollutants, may modify the CF phenotype. Such findings support the great importance of even more aggressive treatment strategies in infants and young children with 3905insT to prevent early lung destruction and development of underweight.

#### Summary

It is concluded that the 3905insT mutation is significantly associated with a severe clinical course, either due to the complete lack of cystic fibrosis transmembrane conductance regulator protein, or to the presence a misgating and misregulating chloride channel. Wide clinical variations within a genetic group sharing the same cystic fibrosis genotype suggest that there other determinants such as modifier genes and environmental factors to be considered.

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